Continuous SSF Experiments with Corn Fiber

Tammy Kay Hayward
June 16, 1995

Experiment Run Dates: January 31-March 17th 1995
Researchers: Tammy Kay Hayward and Kevin M. Connors
Oral Presentation: Amoco-NREL TSC March 1995 (tkh)
Amoco CRADA Bench Scale Research Director: Christos Hatzis
Continuous SSF System Director: George Philippidis
Lab Book Reference: 1651 001-007.

Objectives: To establish a continuous SSF steady state using Corn Fiber and collect data. Experiment represents the first attempt to use a non-hardwood feedstock in the system. To test the effect of dilution rate and enzyme loading on ethanol yield in continuous mode.

Materials and Methods:

Method of Operation: There were two feed vessels, the first one contained the corn fiber and CSL in a pump race, the second feed vessel contained amylase and cellulase enzymes. The yeast maintained their population in the New Brunswick Bioflo III reactor at the set dilution rates. Addition of feeds were coordinated by the same timer on a pulse feed basis. Fermentation product flowed out of the system via a 1/2" port in the side of the reactor. This overflow port insured a constant reactor volume of 1.2 liters. The spent slurry was collected in a sterile container and its weight was displayed by a Ohaus balance and recorded at the time of sampling. See Figure 1 "Schematic of the Continuous System". Whole slurry samples were removed via bottom sample port on the reactor. Sterile air was introduced in the head space of the vessel in order to provide enough gas to meet the minimum flow requirements of the mass spectrometer. The off gas was analyzed by the Fisons VG Prima 600 mass spectrometer for carbon dioxide. This CO2 measurement is one of the best ways to assess the health of the fermentation and prevent wash out. Rotometers monitored the flow of air and off-gas, the Bioflow's flow controller served to hold the flow constant.

Residence Times: A test run using the corn fiber solution was conducted to determine the amount of slurry per discharge and calibrate the timer accordingly. Actual slurry discharge time was fixed at 0.4 seconds throughout the run. Flow is controlled by varying the rest time interval (how long between discharges). See Figure 2 "Tests on Delivery" Enzyme feed was also controlled by the timer. Discharge time remained fixed at 9 seconds throughout the run. Enzyme flow was controlled by adjusting the speed of the enzyme pump. Each time the enzyme loading was changed, the pump was isolated and tested by measuring the volume in microliters per 9 second discharge. The speed was fine tuned until the desired volume was obtained. For the first dilution rate the master timer was set to discharge every 36 minutes. The goal was a 3 day residence time. Three days was the longest residence time the PDU could currently operate at. The second dilution rate was set to discharge every 24 minutes. The residence time was shortened to 2 days. The overflow balance is used to determine the actual through put of the system. The overflow method accounts for clogs, mechanical failures and other disturbances to the system.

Corn Fiber: The substrate used in this experiment was the corn fiber sent to NREL by AMOCO in December of 1994. Material from bucket #11 was stirred and then diluted in D.I. water to form a final feed concentration of 40 w/w %. This was the maximum

concentration that the magnetic stir bar in the feed vessel would tolerate. The solution was neutralized with lime to pH 5. Overliming was not performed. CSL was added to the feed and then the ECF and CSL mixture was autoclaved for 30 minutes.

CSL: The nutrient source employed was 1% v/v Grain Products Corporation Corn Steep Liquor. This CSL is a very thick mixture containing solids. Filter sterilization of the raw CSL proved difficult. So, a 10% dilution of the CSL in DI water was adjusted to pH 5 with ammonium hydroxide, and autoclaved for 30 minutes. This autoclaved stock solution was then filter sterilized and added to the feed flasks prior to autoclaving.

Cellulase and Amylase: The PDU lot of CPN was used as the cellulase enzyme. The activity of the pure, sterile filtered, cellulase was measured by Bill Adney and determined to be 70 FPU per mL. The glucoamylase was a *A. niger* preparation from Sigma. The activity of the sterile filtered, enzyme as stated on the bottle is 6100 units per mL. The amylase enzyme is suspended in one molar glucose and the cellulase enzyme is in 300 g/L sucrose, both have ramifications on the ethanol yield. The two enzymes were mixed together in the enzyme reservoir to achieve a 10 FPU to 100 amylase unit ratio. The recipe was 102.9 mL CPN cellulase and 3.9 mL of Sigma amylase. Enzyme loadings were based on the composition of the corn fiber available at the time of the experiment.

Yeast: The organism used in this experiment was from a plate given to NREL by Ray Bigelis (AMOCO) in December of 1994. A freeze back of this culture was performed. The vials were stored in the new -75 C freezer. A two stage YPD (1% yeast extract, 2% peptone, 2% dextrose) inoculum grown at 34°C was prepared from a vial of the parent strain Labatt 1400. A 10% v/v inoculum was then used to start the batch SSF. No adaptation to the pretreated corn fiber was performed. The yeast maintained its population in continuous mode.

SSF Conditions: The SSF was run at 34°C, 150 rpm, 40 w/w% ECF, with 1% v/v CSL at pH 5 with cellulase and amylase enzymes and head space air. corn fiber. CSL and enzymes were added on a semi-continuous basis and spent slurry containing ethanol was removed by gravity on a constant basis.

<u>Experimental Design:</u>

1. Establish Steady State with ECF, 3 day residence time, 10 FPU/100 units
2. Decrease residence time to 2 days
3. Double the enzyme loading (20 FPU/200 units)
4. Half the original enzyme loading (5 FPU/50 units)

<u>Results:</u>

Established Steady States : The first run with corn fiber and Labatt 1400 yeast in the continuous system was very successful. The run lasted 940 hours, at which time it was deliberately shut down. Under all tested conditions, ethanol was produced, the pH remained constant without vessel pH control, the yeast maintained its population and the glucose remained low. See figure 3 "Continuous SSF . The weight of the overflow slurry was plotted over time, then the actual residence times were calculated based on the slope of the resulting regression lines. The first target was a three day residence time. The calculated value was 75 hours (3 days and 3 hours) for the first setting. The second target was 2 days. The calculated value was determined to be 56 hours or 2 days and 8 hours. Both calculations represent very tight

lines with r-squared values of 0.9974 and 0.9975. See figure 4 "Overflow Balance Data". The compositional analysis at the time of start-up, showed approximately 6% cellulose in the wet slurry. Based on this number the experiments targeted 10 FPU, 20 FPU and 5 FPU. More recent compositional analysis of the corn fiber showed a different cellulose number and a different oligomeric number, changing the enzyme loadings. The actual enzyme loadings were 25 FPU/200 units, 50 FPU/400 units and 12.5 FPU/ 100 units.

A steady state was established under the first set of conditions, 75 hour residence time and 25 FPU/200 units of cellulase and amylase enzymes.

Decreased residence time to 2 days: The residence time was actually decreased to 2 days and 8 hours (56 hours). Ethanol concentration in the vessel remained constant, thus volumetric conversion of the substrate increased. This is also validated by the mass spectrophotometer data which shows a higher volumetric production of carbon dioxide. Flow of carbon dioxide averaged 25 cc/day with the 75 hour residence time and 42 cc/day with the 56 hour residence time. See figure 5 "Off Gas Measurements on Continuous SSF".

Doubled the enzyme: The dilution rate remained the same and the enzyme feed rate was increased to deliver double the amount of cellulase and amylase. At this higher loading of 50 FPU/400 units, ethanol production did increase. Averaged steady state ethanol concentration increased from 14.5 to 15.1 g/L. Unfortunately, both enzyme loadings are saturating and the increase simply represents the additional amounts of glucose and sucrose supplied unavoidable components of the enzyme preparations.

Halved the enzyme: At the time of the experiment, it was believed that the new operational condition was 5 FPU as the enzyme pump was lowered to deliver half the volume of enzyme deliver in batch and the first steady state. Instead, 12.5 FPUs of cellulase and 100 units of amylase were delivered based on the actual composition of the extruded corn fiber. There was a drop in the average steady state ethanol concentration from 15.2 to 12.9 g/L. Also the detected flow of ethanol in the gas phase decreased from 950 to 800 PPM/day. Microscopic observations of the SSF began to include a wide variety of yeast morphologies. No pseudohyphae were observed, however there were small and medium and large cells. A YPD plate of the SSF slurry showed 2 main colonies. With the specter of a possible yeast contaminant present with the Labatt 1400, it was decided to shut down the SSF. A 24 hour fermentation was conducted on D5A, Labatt 1400 and the culture from the ECF continuous. Cultures were grown in two flasks, one with YPD and the other with YPX. Ethanol production and growth only occurred on YPD. Dry cell weight for all three cultures were 3.8 g/L. Ethanol concentrations were also similar at 9.08, 9.14, and 8.36 g/L.

Comparison of Steady States

	Average Ethanol (g/L)	Std dev Ethanol	% Theoretical Yield (C6)	Hours of SSF	Kilograms of Spent Slurry
3 day Res Time 25/200	14.4	0.47	76.7	237	3.53
2 day Res Time 10/100	14.4	0.58	77.0	167	3.55
Double enzyme 20/200	15.2	0.40	81.0	168	2.81
Low enzyme 12.5/50	12.9	0.86	69.0	228	4.67

Also see Figure 6, "Trend Lines for Continuous SSF" to see a graphical representation of the four above steady states.

At the time of shut down, solids and liquids from the harvested vessel were analyzed by the CAT task. The mass balance Excel spreadsheet developed in the first corn fiber study, "Preliminary Experiments , April 1995" was used to check the mass balance on this last sample from the continuous. Two separate scenarios were performed because the solids analysis from the material harvested had a very poor mass balance closure.

Mass Balance Scenario One: The analysis from the solids and liquids actually taken from the continuous vessel were used in the Excel sheet. The mass balance on the solid material is at 75-77%, in other words, 25% of the solid is not accounted for after determining glucose, xylose, galactose, arbinose, mannose, klason lignin, acid-soluble lignin and ash. The analysis was performed again on this solid. Both analyses produced similar poor closures. This analysis is #95-057. The excel sheet shows a conversion of 18.79% on the lignin. Ethanol yield is 32.68 grams per 100 grams of C6 sugars converted. Ethanol process yield is 51.1% of theoretical in the SSF unit operation. Cellulose conversion is at 95.7%. The overall carbon recovery is 89.76%. Again a significant portion of the six carbon sugars remain in the liquor as unconverted oligomers. See Figure 7 "SSF Carbon Balance: continuous SSF on 40% cat95-057".

Mass Balance Scenario Two: The analysis of the solid residue from the shake flask study had closed to 89%. The main difference is the higher reported lignin. The analysis from this solids was placed in the excel sheet. The lignin conversion improved from 18.79 to -2.86. The overall carbon recovery improves to 94.71%. Ethanol remains unchanged. See figure 8 "SSF Carbon Balance:continuous SSF on 40% cat95-020". Also see Appendix 2, "CAT Task reports".

Conclusions:

The first attempt at continuous fermentation of Corn Fiber was very successful. The run lasted 940 hours at which time it was deliberately shut down. The feed concentration of Corn Fiber was 40 % w/w. This was the maximum solids loading that the magnetic stir bar in the feed jar could tolerate. The following steady state conditions were tested: 25 FPU/200 units 75 hour residence time, 25 FPU/200 units 56 hour residence time, 50 FPU/400 units 56 hour residence time, 12.5 FPU/100 units 56 hour residence time. The percent theoretical ethanol yields based on total six carbon sugars were 77, 77, 81 and 69 respectively. Unfortunately the enzyme loadings actually used in the experiment were all saturating for this substrate and thus may explain the relatively small differences in ethanol yield of the various tested conditions.

Glucose levels remained under 2 g/L through out the entire 940 hour run. The pH remained stable without vessel pH control (extruded corn fiber feed was adjusted to pH 5 with lime before autoclaving). The yeast maintained their population in the fermentor at the tested dilution rates of 0.013 and 0.017 h^{-1} which correspond to residence times of 75 and 56 hours. Continuous inoculation was not needed.

Based on plate cell counts, at the end of the run, an equal number of foreign yeast accompanied the Labatt 1400 parent strain. No other organisms were observed on the plates or under the microscope through out the run. Despite the presence of 10 g/L free xylose during the SSF, bacteria or other xylose utilizing organisms were not detected at any time.

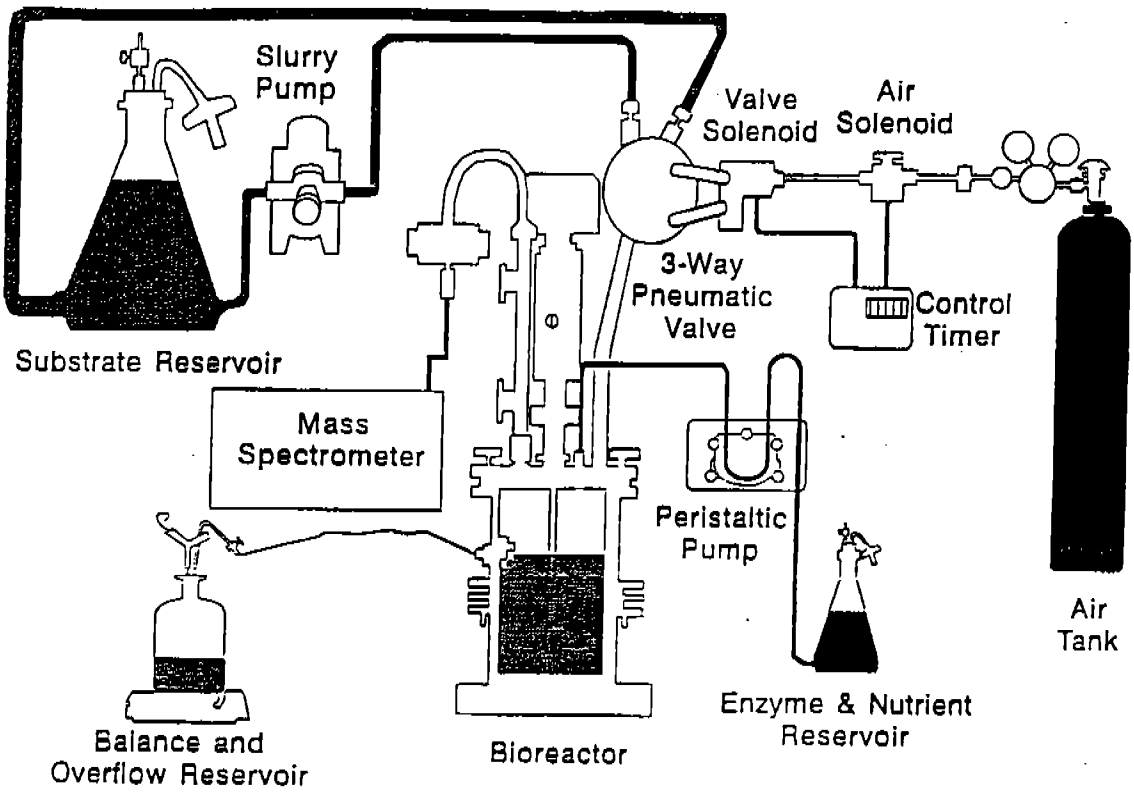
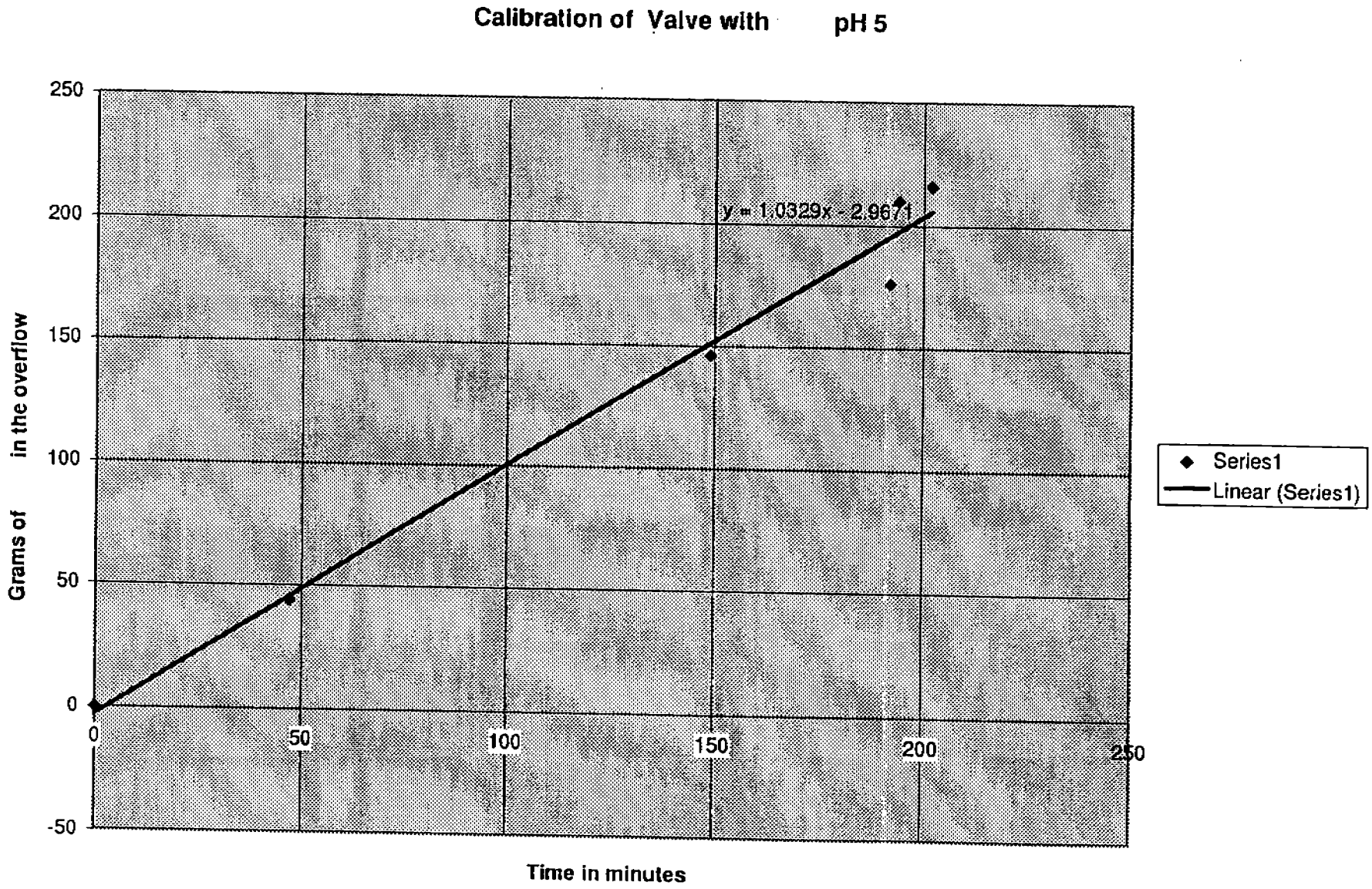
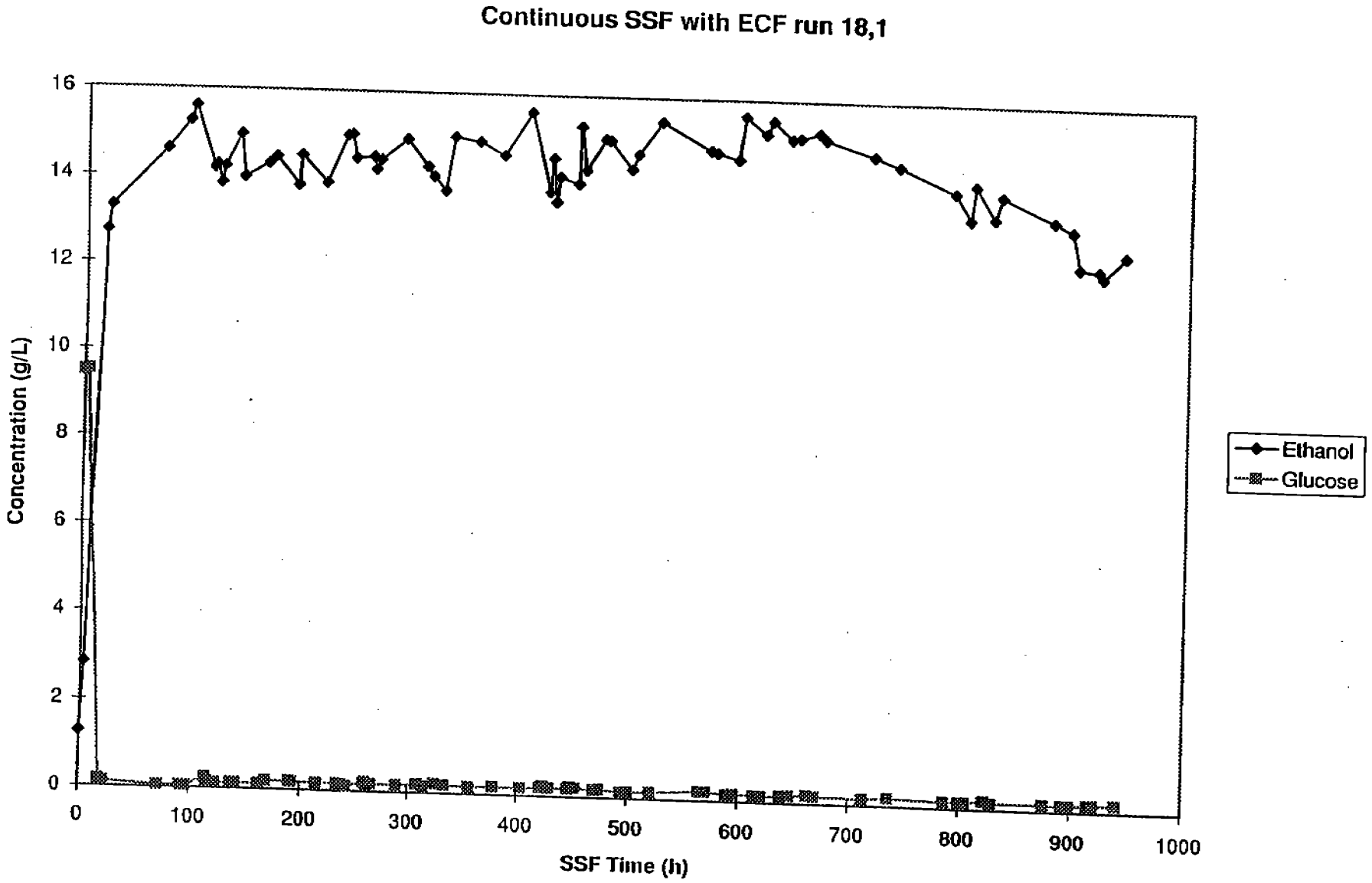


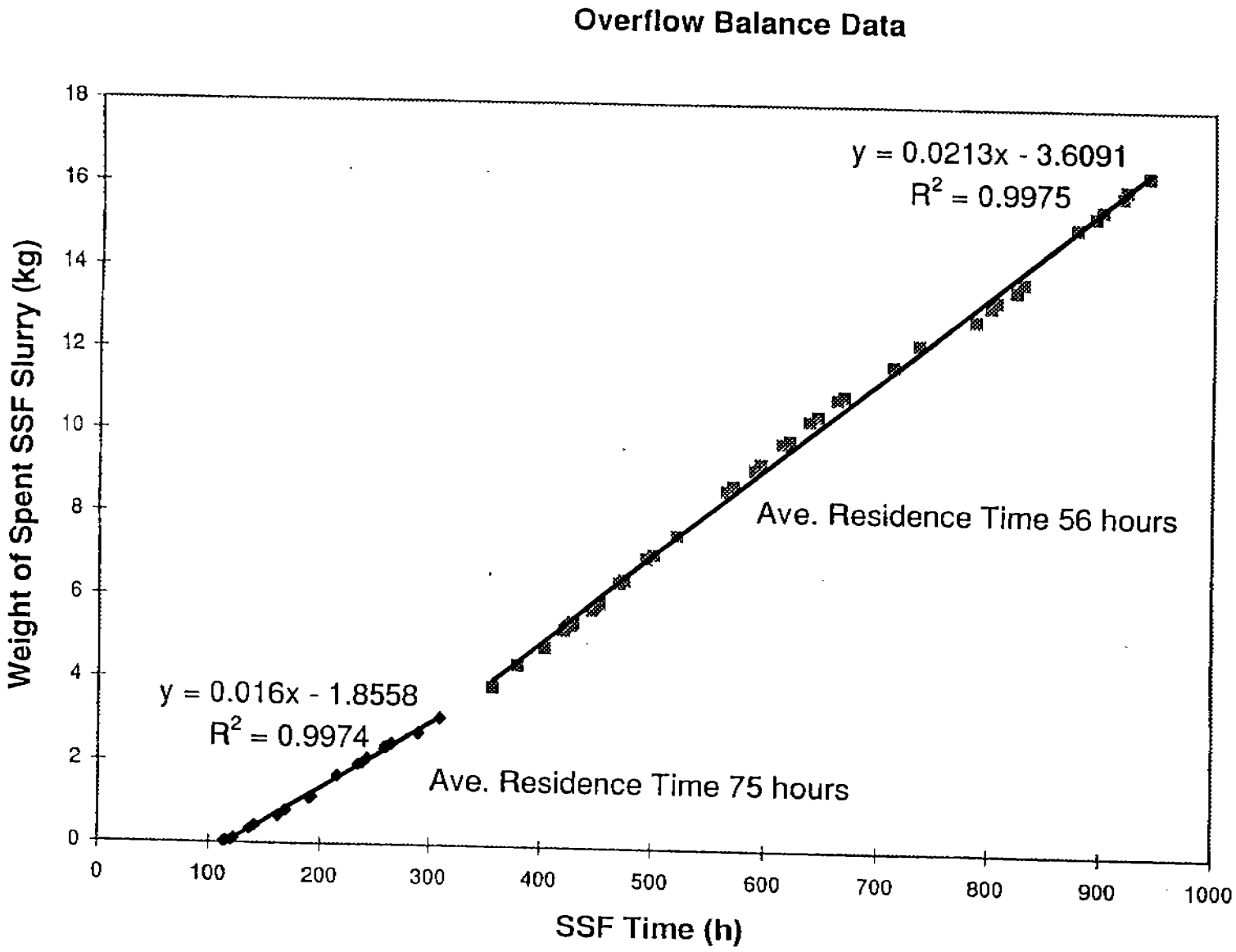
FIGURE 1. SCHEMATIC OF THE CONTINUOUS SSF SET-UP.


Calibration of Valve with pH 5
y = 1.0329x - 2.9671
Grams of in the overflow
Time in minutes
250
200
150
100
50
0
-50
0
50
100
150
200
250
Series1
Linear (Series1)

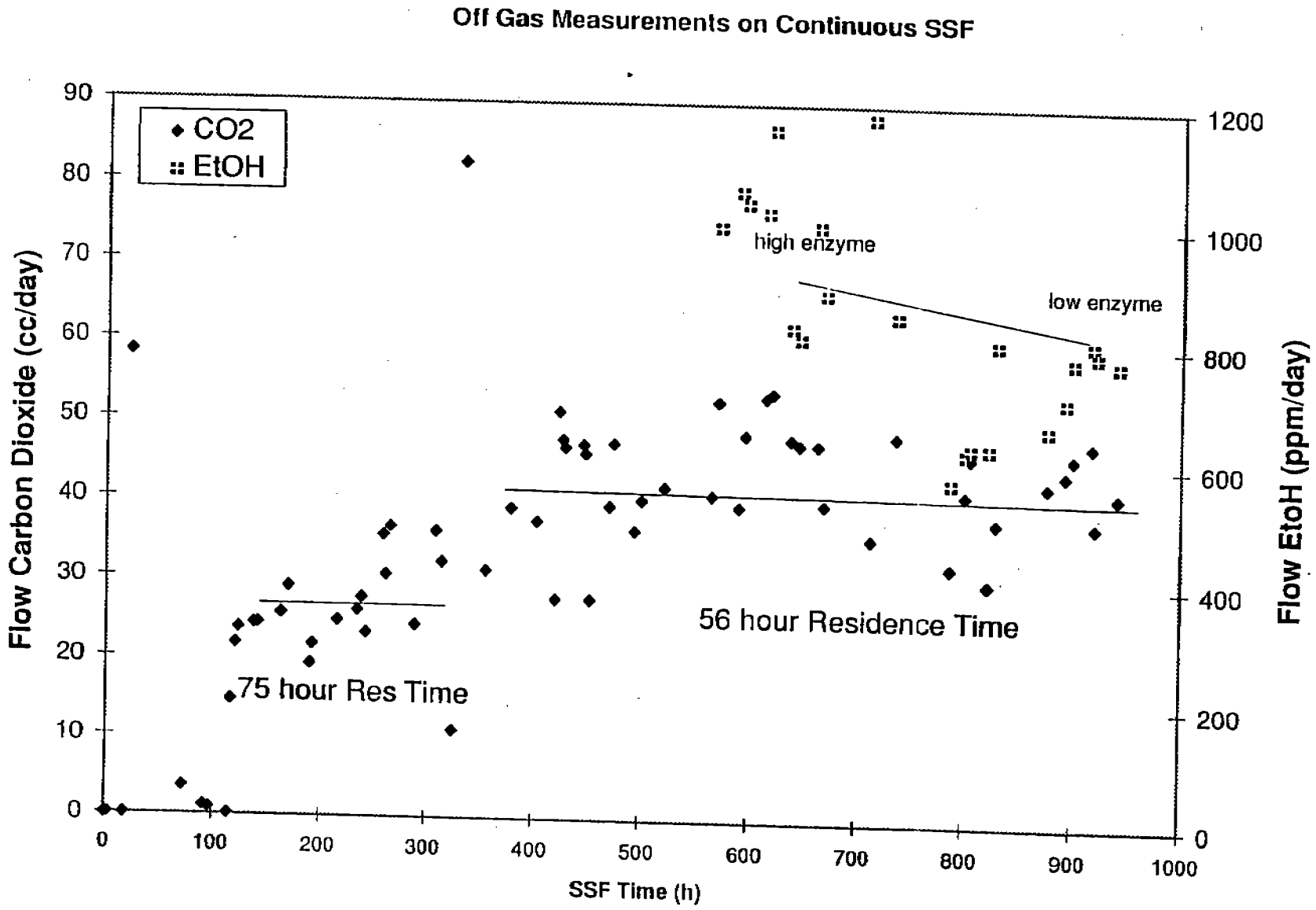
First run with Corn Fiber. SSF ran in continuous mode for over 900 hours. Concentrations of glucose remain low. Ethanol levels are lower towards the end of the run when the enzyme loading was the lowest.



Continuous SSF with ECF Run 18, 1 Spent SSF slurry as it falls into the overflow container over time. Used to confirm flowrates and residence times.

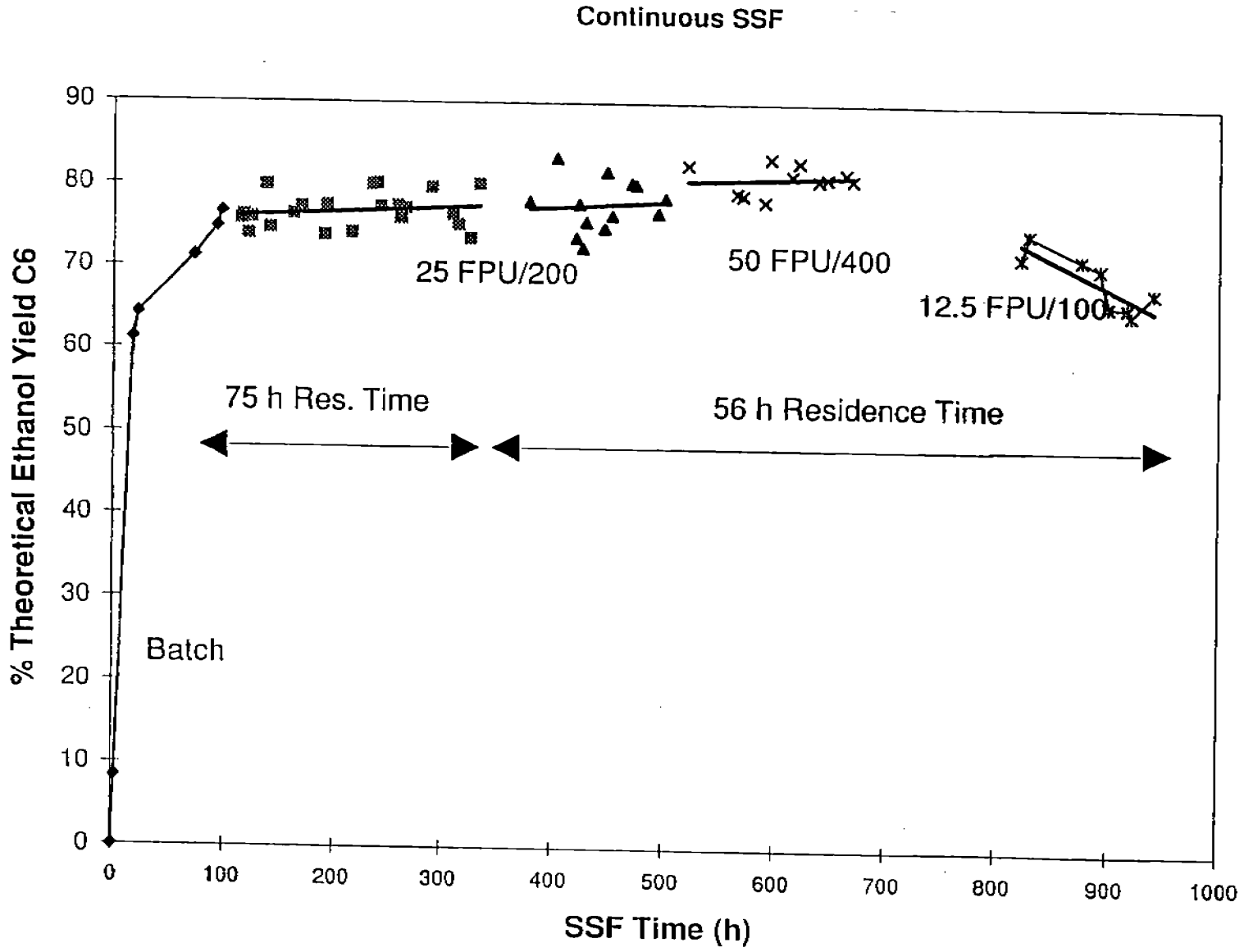


Rate of Carbon Dioxide Production in Continuous SSF at two Residence Times



Tammy Kay Hayward

Trendlines of various steady states with Corn Fiber in Continuous SSF Mode. Parent Strain Labatt 1400. Enzyme loadings were 10, 20 and 5 FPU based on January 1995 cellulose composition numbers.



'25/95

SSF CARBON BALANCE: Continuous SSF on 40% Corn Fiber (caf95-057)

Sample: :
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Ugn in (%):	30.83	~~39.61~~ 54.09
Insoluble Solids (%):	3.50	1.40

Cellulose Conversion:	95.7%
Overall C6-Sugar Conversion:	80.0%
Overall C5-Sugar Conversion:	6.0%
Ethanol Process Yield (% theor):	51.1%
Ethanol Metabolic Yield (% theor):	63.9%

Carbon Balance: SSF

Component	Carbon In: In Solids (% dry wt)	(C-mole/Kg Sl)	(% Total In)	In Liquor (g/L)	(C-mole/Kg Sl)	(% Total In)	Total (C-mole/Kg Sls)	Carbon Out: In Solids (% dry wt)	(C-mole/Kg Sl)	(% Total Out)	In Liquor (g/L)	(C-mole/Kg Sl)	(% Total Out)	Total (C-mole/Kg Sl)	Conversion (In-Out)/In (%)	Yield g product/ 100 g C6 con
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000		
Glucose	51.27	0.598	*50.7*	18.05	0.580	*49.3*	1.178	5.51	0.026	*13.7*	4.91	0.161	*86.3*	0.187	84.13	
Galactose	1.42	0.017	*11.1*	4.12	0.133	*88.9*	0.149	0.19	0.001	*1.0*	2.81	0.092	*99.0*	0.093	37.51	
Mannose	0.12	0.001	*1.7*	2.55	0.082	*98.3*	0.083	0.57	0.003	*100.0*	0.00	0.000	*0.0*	0.003	96.81	
Xylose	9.02	0.105	*14.7*	19.04	0.612	*85.3*	0.717	0.89	0.004	*0.6*	20.39	0.670	*99.4*	0.674	6.06	
Arabinose	4.54	0.053	*12.2*	11.80	0.379	*87.8*	0.432	0.27	0.001	*0.3*	12.35	0.406	*99.7*	0.407	5.87	
Lignin	30.83	0.516	*65.4*	5.93	0.273	*34.6*	0.789	54.08	0.362	*56.5*	5.92	0.279	*43.5*	0.641	18.79	
Ethanol				1.00	0.042		0.042				12.20	0.522		0.522		32.68
Cell Mass				0.20	0.008		0.008				2.24	0.088		0.088		5.95
Carbon Dioxide												0.254		0.254		33.08
Glycerol				0.08	0.002		0.002				0.55	0.018		0.018		1.39
Acetic Acid				1.61	0.052		0.052				3.02	0.099		0.099		4.20
Lactic Acid				0.49	0.016		0.016				2.92	0.096		0.096		7.10
Succinic Acid				0.84	0.027		0.027				1.68	0.056		0.056		2.50
Total	*90.29*	*1.289*	*36.9*		*2.206*	*63.1*	*3.496*	*60.74*	*0.396*	*12.6*		*2.741*	*87.4*	*3.138*		*86.90*

C-Recovery: 89.76%

SSF CARBON BALANCE: Continuous SSF on 40% Corn Fiber (cal95-020)

Sample:
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	30.83	79.61
Insoluble Solids (%):	3.50	1.40

Cellulose Conversion:	96.5%
Overall C6 Sugar Conversion:	80.4%
Overall C5 Sugar Conversion:	5.2%
Ethanol Process Yield (% theor):	51.1%
Ethanol Metabolic Yield (% theor):	63.6%

Carbon Balance: SSF

Component	Carbon In: In Solids (% dry wt)	(C-mole/Kg Sl)	(% Total In)	In Liquor (g/L)	(C-mole/Kg Sl)	(% Total In)	Total (C-mole/Kg Sl)	Carbon Out: In Solids (% dry wt)	(C-mole/Kg Sl)	(% Total Out)	In Liquor (g/L)	(C-mole/Kg Sl)	(% Total Out)	Total (C-mole/Kg Sl)	Conversion (In-Out)/In (%)	Yield g product/ 100 g C6 con
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000		
Glucose	51.27	0.598	50.7	18.05	0.580	49.3	1.178	4.43	0.021	11.4	4.91	0.161	88.6	0.182	84.56	
Galactose	1.42	0.017	11.1	4.12	0.133	88.9	0.149	0.54	0.003	2.7	2.81	0.092	97.3	0.095	36.42	
Mannose	0.12	0.001	1.7	2.55	0.082	98.3	0.083	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.00	
Xylose	9.02	0.105	14.7	19.04	0.612	85.3	0.717	2.43	0.011	1.7	20.39	0.670	98.3	0.681	5.06	
Arabinose	4.54	0.050	12.2	11.80	0.379	87.8	0.432	0.55	0.003	0.6	12.35	0.406	99.4	0.408	5.56	
Lignin	30.83	0.516	65.4	5.93	0.273	34.6	0.789	79.61	0.533	65.6	5.92	0.279	34.4	0.812	-2.86	
Ethanol				1.00	0.042		0.042				12.20	0.522		0.522		32.61
Cell Mass				0.20	0.008		0.008				2.24	0.088		0.088		5.92
Carbon Dioxide												0.254		0.254		32.90
Glycerol				0.08	0.002		0.002				0.55	0.018		0.018		1.38
Acetic Acid				1.61	0.052		0.052				3.02	0.099		0.099		4.18
Lactic Acid				0.49	0.016		0.016				2.92	0.096		0.096		7.06
Succinic Acid				0.84	0.027		0.027				1.68	0.056		0.056		2.49
Total	90.29	1.289	36.9		2.206	63.1	3.496	86.71	0.570	17.2		2.741	82.8	3.311		86.44

C-Recovery:	94.71%

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-057 | Page 1 of 1

Project Title: Continuous SSF (ET60)

NREL In-House	Current Subcontractor	CRADA	Other
☐	☐	☒	☐

Name of Project Contact Person: Tammy Kay Hayward	Date Work Completed: Revised report issued 5/18/95
NREL Notebook: 1561, p037	Date Samples Delivered: 3/17/95
Samples from Feedstock Lot No.: N/A	Actual Hours Spent: 2
Summary of Requested Work: Complete compositional analysis.	Proposed Approach: Standard LAPs by validated outside laboratory.

Work Required: Sample Prep ☒ Acid Digest ☒ HPLC ☒ YSI ☐ GC ☐ Other:

Results and Comments ☐ % As Received ☒ % Dry Weight ☐ mg/mL ☐ Other:

	Sample		TS	G	X	GA	A	M	LKL	LAS	AT	MB		
1	Continuous Final Pt. Autoclaved washed solids, 95-057-644, initial (March) analysis	ave	35.11	15.67	2.53	0.56	0.77	1.62	48.74	5.34	2.07	75.12		
		sd	0.12	0.57	0.00	0.08	0.02	0.10	0.87	0.09	0.01	---		
	Continuous Final Pt. Autoclaved washed solids, 95-057-644, reanalyzed (May)	ave	35.16	17.59	2.82	0.57	0.92	1.49	48.64	5.35	2.12	77.09		
		sd	0.21	0.22	0.10	0.02	0.02	0.05	0.16	0.12	0.02	---		
		ave												
		sd												
		ave												
		sd												
		ave												
		sd												
		ave												
		sd												

A=arabinose; AC=acetic acid; AT=total ash; ET=ethanol; FA=formic acid; FL=furfural; G=glucose; G-YSI=glucose by YSI; GA=galactose; GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LA=lactic acid; LAS=acid soluble lignin; LKL=Klason lignin; M=mannose; MB=mass balance. [(G+GA+M)x0.90 + (X+A)x0.88 +LKL + LAS + AT]; n/a=not applicable; nd=not detected; nr=not requested; P=protein; SA=succinic acid; TS=total solids; TDS=total dissolved solids; X=xylose; *=Not enough sample to run in duplicate.

Name(s) of CAT Staff Working on Project: Larry Brown

Reviewed by: Tina Ehrman

Tina Ehrman 5-19-95

CC: Christos Hatzis

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-020 | Page 1

Project Title: Extruded Corn Fiber SSFs ECF1 (ET60)

NREL In-House ☐ Current Subcontractor ☐ CRADA ☒ Other ☐

Date Samples Delivered: 2/9/95

Date Work Promised: 2/14/95

Name of Project Contact Person: Tammy Kay Hayward

Date Work Completed: 2/15/95

NREL Notebook: #1561, p017, #1382, p108

Estimated Hours Required: 4

Samples from Feedstock Lot No.: n/a

Actual Hours Spent: 4

Summary of Requested Work: Complete compositional analysis, protein content.

Proposed Approach: Standard Laps by validated outside laboratory, protein content by in house CHN analysis.

Work Required: Sample Prep ☒ NDF/ADF ☐ Acid Digest ☒ HPLC ☒ YSI ☐ GC ☐ Other:

Results and Comments ☐ % As Received ☒ % Dry Weight ☐ Other: 79.61

	Sample		TS	G	X	GA	A	M	LKL	LAS	AT	MB
1	Autoclaved SSF solid residue	ave	30.60	4.43	2.43	0.54	0.55	0.0	73.80	5.81	1.53	89.09
		sd	0.21	0.33	0.11	0.02	0.07	0.0	0.29	0.23	0.03	
		ave										
		sd										
3		ave										
		sd										
4		ave										
		sd										
5		ave										
		sd										
6		ave										
		sd										
7		ave										
		sd										

A=arabinose; AC=acetate; AD=detergent ash; AT=total ash; C=mass % carbon; CE=cellulose; ET=ethanol; FL=furfural; G=glucose; GA=galactose; H=mass % hydrogen; HC=hemicellulose; L=detergent lignin; LAS=acid soluble lignin; LKL=Klason lignin; M=mannose; N=mass % nitrogen; nd=not detected; nr=not requested; P=protein; TS=total solids; UA=uronic acids; X=xylose; *=calculated from nitrogen measured by CHN

Name(s) of CAT Staff Working on Project: Larry Brown, Ruiz — Larry Brown, Ruy Ruiz

CAT Task Leader: Tina Ehrman — Tina Ehrman

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-058 | Page 1 of 1

[redacted]ct Title: Continuous SSF (ET60)

NREL In-House ☐ | Current Subcontractor ☐ | CRADA ☒ | Other ☐

Name of Project Contact Person: Tammy Kay Hayward

Date Work Completed: 3/31/95

NREL Notebook: 1561 p 038. #1385, p034

Date Samples Delivered: 3/17/95

Samples from Feedstock Lot No.: N/A

Actual Hours Spent: 4

Summary of Requested Work: Monomeric sugars in sample as received organic acids, HMF, furfural, ethanol in sample as received; total sugars in sample after 4% hydrolysis

Proposed Approach: Standard LAPs by validated outside laboratory and by in house analyst.

Work Required: Sample Prep ☒ | Acid Digest ☒ | HPLC ☒ | YSI ☐ | GC ☒ | Other:

Results and Comments ☐ % As Received ☐ % Dry Weight ☒ mg/mL ☐ Other:

Sample		G	X	GA	A	M	SA	LA	GLY	AC	HMF	FL	ET
[redacted] Continuous final pt filter sterile [redacted]uid, 95-058-645, as received	ave	1.53	10.94	1.65	9.13	0.00	1.68	2.92	0.55	3.02	0.00	0.07	12.2
	sd	0.02	0.06	0.02	0.06	0.00	0.01	0.03	0.01	0.03	0.00	0.01	—
95-058-645, after 4% acid hydrolysis	ave	4.91	20.39	2.81	12.35	0.00	—	—	—	—	—	—	—
	sd	0.00	0.00	0.00	0.01	0.00	—	—	—	—	—	—	—
	ave												
	sd												
	ave												
	sd												
	ave												
	sd												
	ave												
	sd												

A=arabinose; AC=acetic acid; AT=total ash; ET=ethanol; FA=formic acid; FL=furfural; G=glucose; G-YSI=glucose by YSI; A=galactose; GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LA=lactic acid; LAS=acid soluble lignin; LKL=Klason lignin; M=mannose; n/a=not applicable; nd=not detected; nr=not requested; P=protein; SA=succinic acid; TS=total solids; TDS=total dissolved solids; X=xylose; *=Not enough sample to run in duplicate.

[redacted]me(s) of CAT Staff Working on Project: Larry Brown, [redacted]vi[redacted]npleton Larry Brown David W Templeton

Reviewed by: Tina Ehrman

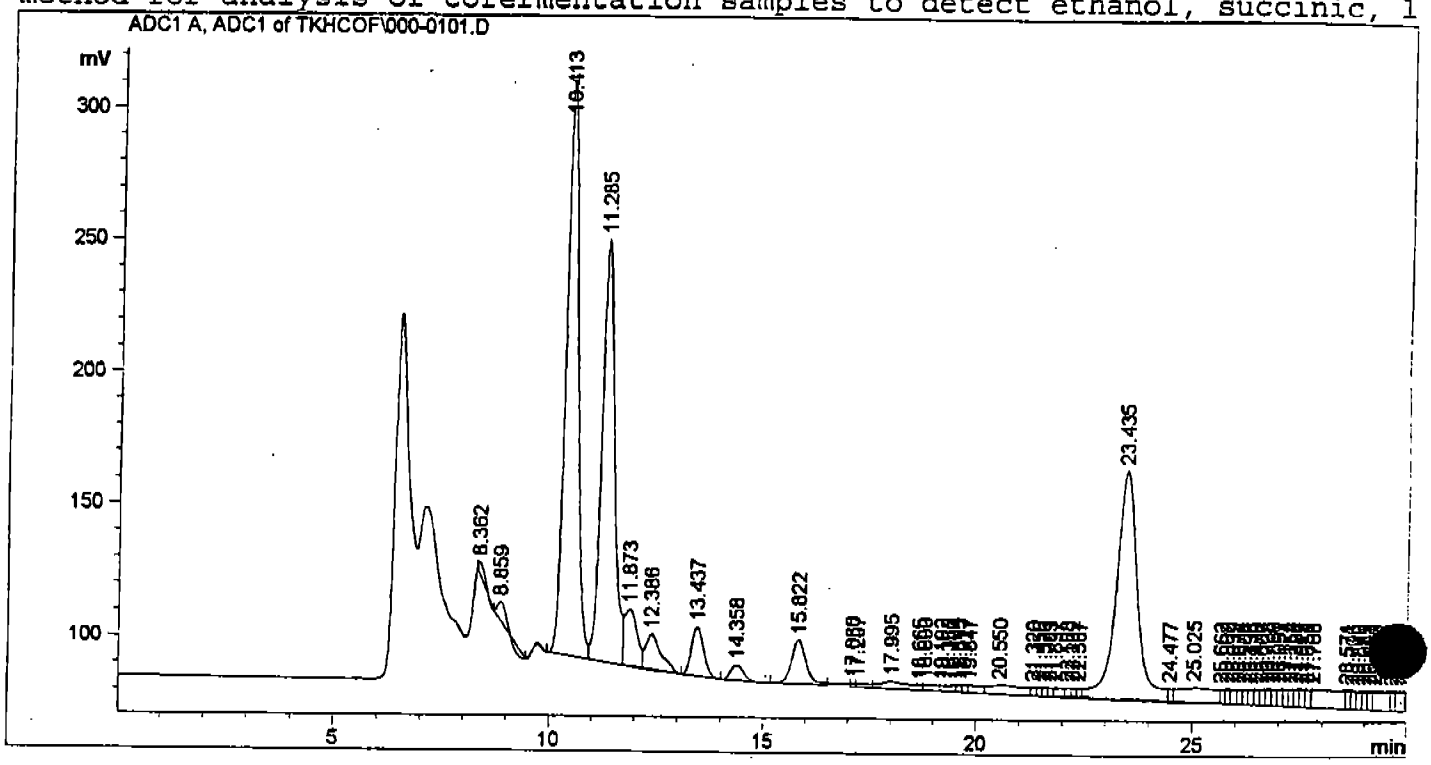
Tina Ehrman

Christos Hatzis

Acq. Method : COFERM.M
Acq. Operator : KENT
Injection Date : 3/2/95 4:40:27 PM
Sample Name : #49

Seq. Line : 1
Vial : 0
Inj : 1
Inj Volume : 10 µl

Sequence File : C:\HPCHEM\1\SEQUENCE\TKHCOF.S
Analysis Method : C:\HPCHEM\1\METHODS\COFERM.M
method for analysis of cofermentation samples to detect ethanol, succinic, l



External Standard Report

Sorted by Signal
Calib. Data Modified : Thursday, March 02, 1995 12:29:20 PM
Multiplier : 1.000000
RF Uncal. Peaks : 1.000000

Signal 1: ADC1 A, ADC1

RT [min]	Type	Area	Amt/Area	Amount [g/L]	Grp	Name
7.872	*	not found	*			Cellobiose
8.362	BV	48.62407	1.00000	48.62407		?
8.859	PV	58.57494	1.00000	58.57494		?
9.750	*	not found	*			glucose
10.413	BV	4291.61719	2.32417e-3	9.97445		xylose
11.285	VV	3324.44800	1.00000	3324.44800		?
11.873	VV	440.38184	1.00000	440.38184		?
12.386	VV	338.45407	2.72906e-3	9.23662e-1		succinic acid
13.437	PV	377.72403	2.88063e-3	1.0880		[illegible] acid
14.358	PV	125.52240	3.34008e-3	4.19255e-1		
15.822	VV	413.54282	4.99691e-3	2.06643		acetic acid

Data File c:\HPCHEM\1\DATA\TKHCOF\000-0101.D Sample Name: #49

RT [min]	Type	Area	Amt/Area	Amount [g/L]	Grp	Name
17.080	VV	10.34459	1.00000	10.34459	?	
17.207	VV	32.14430	1.00000	32.14430	?	
17.995	VV	136.28113	1.00000	136.28113	?	
18.665	VV	16.45382	1.00000	16.45382	?	
18.800	VV	50.93551	1.00000	50.93551	?	
19.192	VV	17.61430	1.00000	17.61430	?	
19.338	VV	18.48275	1.00000	18.48275	?	
19.484	VV	16.61640	1.00000	16.61640	?	
19.615	VV	20.87583	1.00000	20.87583	?	
19.727	VV	22.68722	1.00000	22.68722	?	
19.847	VV	62.83945	1.00000	62.83945	?	
20.550	VV	195.95222	1.00000	195.95222	?	
21.320	VV	24.77899	1.00000	24.77899	?	
21.456	VV	24.07818	1.00000	24.07818	?	
21.576	VV	27.12610	1.00000	27.12610	?	
21.703	VV	25.96850	1.00000	25.96850	?	
21.837	VV	55.04248	1.00000	55.04248	?	
22.108	VV	28.25650	1.00000	28.25650	?	
22.232	VV	28.97863	1.00000	28.97863	?	
22.367	VV	29.64168	1.00000	29.64168	?	
23.435	VV	2993.80420	5.59723e-3	16.75701		ethanol
24.477	VV	40.17390	1.00000	40.17390	?	
25.025	VV	354.63028	1.00000	354.63028	?	
25.662	VV	39.16637	1.00000	39.16637	?	
25.802	VV	42.83253	1.00000	42.83253	?	
25.939	VV	46.90511	1.00000	46.90511	?	
26.074	VV	46.01423	1.00000	46.01423	?	
26.204	VV	46.87158	1.00000	46.87158	?	
26.338	VV	47.70601	1.00000	47.70601	?	
26.470	VV	48.64779	1.00000	48.64779	?	
26.607	VV	49.64444	1.00000	49.64444	?	
26.738	VV	50.64853	1.00000	50.64853	?	
26.866	VV	51.55343	1.00000	51.55343	?	
26.991	VV	52.29885	1.00000	52.29885	?	
27.124	VV	48.19789	1.00000	48.19789	?	
27.254	VV	52.51472	1.00000	52.51472	?	
27.384	VV	53.71774	1.00000	53.71774	?	
27.515	VV	52.57881	1.00000	52.57881	?	
27.651	VV	53.60887	1.00000	53.60887	?	
27.788	VV	335.41895	1.00000	335.41895	?	
28.571	VV	55.85745	1.00000	55.85745	?	
28.706	VV	50.86274	1.00000	50.86274	?	
28.840	VV	55.35005	1.00000	55.35005	?	
28.971	VV	56.70454	1.00000	56.70454	?	
29.103	VV	56.90847	1.00000	56.90847	?	
29.235	VV	174.96999	1.00000	174.96999	?	
29.632	VV	58.90648	1.00000	58.90648	?	
29.765	VV	59.47293	1.00000	59.47293	?	
29.897	VBA	33.00539	1.00000	33.00539	?	

Totals : 6863.52441

1 Warnings or Errors :

Warning : Calibrated compound(s) not found